Effect of Amphetamine on Dopamine D2 Receptor Binding in Nonhuman Primate Brain: A Comparison of the Agonist Radioligand [^{11}C]MNPA and Antagonist [^{11}C]Raclopride

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ABSTRACT PET measurements of stimulant-induced dopamine (DA) release are typically performed with antagonist radioligands that bind to both the high- and low-affinity state of the receptor. In contrast, an agonist radioligand binds preferentially to the high-affinity state and is expected to have greater sensitivity to DA, which is the endogenous agonist. [^{11}C]MNPA, (*R*)-2-CH_3O-*N*-*n*-propylnorapomorphine (MNPA), is a D_2 agonist radioligand with subnanomolar affinity to the D_2 receptor. The aim of the present study is to assess and compare the sensitivity of the agonist radioligand [^{11}C]MNPA and antagonist radioligand [^{11}C]raclopride to synaptic DA levels. Four cynomolgus monkeys were examined with [^{11}C]MNPA and [^{11}C]raclopride (16 PET measurements with each tracer) at baseline and after pretreatment with various doses of amphetamine. The effect of amphetamine was calculated by the change in binding potential (BP) analyzed with the multilinear reference tissue model (MRTM2). Amphetamine caused a reduction in [^{11}C]MNPA BP of 4% at 0.1, 23% at 0.2, 25% at 0.5, and 46% at 1.0 mg/kg. [^{11}C]Raclopride BP was reduced to a lesser extent by 2% at 0.1, 16% at 0.2, 15% at 0.5, and 23% at 1.0 mg/kg. The data were used to estimate the in vivo percentage of high-affinity state receptors to be ~60%. These results demonstrate that [^{11}C]MNPA is more sensitive than [^{11}C]raclopride to displacement by endogenous DA, and that it may provide additional information about the functional state of the D_2 receptor in illnesses such as schizophrenia and Parkinson's disease. **Synapse 59:260–269, 2006.** Published 2006 Wiley-Liss, Inc.†

INTRODUCTION

PET neuroreceptor imaging can be combined with a pharmacological challenge to measure indirectly the extracellular levels of neurotransmitters. That is, the competition of endogenous neurotransmitter and PET radioligand for binding to the target receptor can be used to assess changes in the transmitter itself. The most extensively studied system to date with this paradigm is that defined by the DA D_2 receptor. Pharmacological challenges that either increase or decrease synaptic concentrations of DA are combined with PET imaging of the D_2 receptor using tracer doses of the radioligand. For example, agents that increase synaptic DA concentrations like amphetamine (AMPH) or methylphenidate have been shown in human and nonhuman primates to decrease D_2 receptor radioligand binding (Carson et al., 1997; Farde and Hall, 1992; Innis et al., 1992; for review

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see Laruelle, 2000; Laruelle et al., 1995; Volkow et al., 1994). Similarly, agents that reduce synaptic DA levels (e.g., reserpine or α-methyl-*p*-tyrosine) decrease competition by the endogenous transmitter and thereby "unmask" D_2 receptors and increase radioligand binding (Ginovart et al., 1997; Laruelle et al., 1997; Verhoeff et al., 2001).

These endogenous competition studies have typically been performed with antagonist radioligands, such as [^{11}C]raclopride. Antagonists of G-protein coupled receptors, such as the D_2 receptor, have equal affinity for receptors in the high (i.e., coupled) and low (i.e., uncoupled) affinity states. In contrast, agonists bind preferentially to the high affinity state (Creese et al., 1984; George et al., 1985; Seeman et al., 1985; Sibley et al., 1983). Since DA is the endogenous agonist for the D_2 receptor, it would more effectively compete with the binding of an agonist, compared with an antagonist radioligand. In addition to being more sensitive to competition by endogenous transmitter, an agonist radioligand for the D_2 receptor will provide in vivo information on the percentage of receptors in the high affinity state, which may well be abnormal in disorders such as schizophrenia (Farde et al., 1987; Laruelle et al., 1996), Parkinson's disease (de La Fuente-Fernandez et al., 2001; Tedroff et al., 1996), bipolar disorder, and unipolar depression (Anand et al., 2000; Parsey et al., 2001).

Several ex vivo (Cumming et al., 2002; Kohler et al., 1981; Ross and Jackson, 1989; Van Der Werf et al., 1983) and in vitro studies (Sibley and Creese, 1983; Sibley et al., 1983) have demonstrated that the agonist radioligand [^{3}H]NPA (norpropylapomorphine) binds preferentially to the high affinity state of D_2 receptors in rodent brain. After AMPH administration, greater inhibition of [^{3}H]NPA binding compared with antagonist radioligands supports that the two radioligands may bind to a separate subpopulation of D_2 receptors. In line with this observation, recent PET imaging studies with D_2 agonist radioligands in animals support preferential labeling of D_2 receptors in the high affinity state (Cumming et al., 2003; Mukherjee et al., 2004; Narendran et al., 2004; Wilson et al., 2005). For instance, the agonist radioligand [^{11}C]NPA has been reported to have a 42% larger decrease in binding after AMPH administration compared with the antagonist [^{11}C]raclopride (Narendran et al., 2004).

Finnema et al. (2005a) recently reported the labeling of the high affinity DA D_2 agonist [^{11}C]MNPA, which is a methoxy analog of NPA. Both NPA and MNPA are highly selective for the D_2 receptor compared with the D_1 receptors (Gao et al., 1990). By using the methylation approach, [^{11}C]MNPA is more easily synthesized than [^{11}C]NPA. In addition, [^{11}C]MNPA has higher affinity for the D_2 receptor compared with [^{11}C]NPA:IC_{50} of 1.02 nM or K_i of 0.17 nM compared with IC_{50} of 4.80 nM or K_i of 0.8 nM, respectively (Gao et al., 1990; Neumeyer et al., 1990). The reported D_1 selectivity of NPA:IC_{50} of 640 nM or K_i of 340 nM and MNPA IC_{50} of 3,340 nM or K_i of 1,780 nM, with a D_2/D_1 K_i ratio of 425 for NPA and 10,500 for MNPA (Gao et al., 1990). In preliminary PET measurements performed in cynomolgus monkeys, high uptake was seen in brain regions known to contain high densities of D_2 receptor, with a maximum striatum to cerebellum ratio of ~2.2. The striatal uptake of [^{11}C]MNPA could be displaced by injection of unlabeled raclopride (1.0 mg/kg), confirming that the striatal binding was associated with saturable sites.

The aim of the present study is to examine whether the striatal uptake of the agonist radioligand [^{11}C]MNPA is more sensitive than the antagonist radioligand [^{11}C]raclopride to stimulant-induced DA release. Cynomolgus monkeys were examined before and after increasing doses of i.v. amphetamine. Finally, the data were used in an attempt to estimate the proportion of D_2 receptors in the high and low affinity states in the anesthetized nonhuman primate.

MATERIALS AND METHODS

Radiochemistry

[^{11}C]raclopride and [^{11}C]MNPA were prepared as described previously (Langer et al., 1999; Finnema et al., 2005a). Briefly, [^{11}C]MNPA was prepared by methylation of (*R*)-2-OH-NPA with [^{11}C]methyl iodide, which is selective to the 2-OH position and confirmed by high-performance liquid chromatography comparison with a standard. The labeling gave an incorporation yield of 75% and a radiochemical purity of >99%.

PET system

Radioactivity in brain was measured with the Siemens ECAT Exact HR 47 system. All acquisitions were acquired in 3D-mode (Wienhard et al., 1994). A three ring detector block architecture gives a 15-cm wide field of view. The transversal resolution in the reconstructed image is about 3.8 mm full width half maximum and an axial resolution of 3.125 mm. The attenuation correction of the data was obtained with the three rotating ^{68}Ge line sources. Raw PET data were then reconstructed using the standard filtered back projection consisting of the following reconstruction parameters: 2-mm Hanning Filter, scatter correction, a zoom factor of 2.17, and a 128 × 128 matrix size (Wienhard et al., 1994). Emission data for [^{11}C]MNPA and [^{11}C]raclopride were collected continuously for 93 min, according to a preprogrammed series of 20 frames starting immediately after i.v. injection of radioligand. The three initial frames were 1 min each, followed by 4 scans of 3 min each and the remaining frames of 6 min.

PET studies

Four cynomolgus monkeys (*Macaca fascicularis*) weighing 3–4 kg were supplied by Astrid Fagraeus Laboratory, Swedish Institute for Infectious Disease Control (SMI), Solna, Sweden. The study was approved by the Animal Research Ethical Committee of the Northern Stockholm Region. Anesthesia was induced and maintained by repeated i.m. injections of a mixture of ketamine hydrochloride (3.75 mg/kg h Ketalar®, Pfizer) and xylazine hydrochloride (1.5 mg/kg h Rompun® Vet., Bayer). The head was immobilized with a fixation device (Karlsson et al., 1993), and body temperature was maintained by Bair Hugger–Model 505 (Arizant Healthcare Inc., MN) and monitored by a rectal thermometer (Precision Thermometer, Harvard Apparatus, MA). Cardiac and respiratory rates were measured every 20 min.

A total of 32 PET measurements were performed over 1.5 years with [^{11}C]MNPA ($n = 16$) and [^{11}C]raclopride ($n = 16$) in four monkeys. A baseline measurement was performed in the morning and a pretreatment measurement in the afternoon of the same day. AMPH was injected i.v. as a 30 s bolus ~20 min before injection of [^{11}C]MNPA or [^{11}C]raclopride. For each dose of AMPH, the same monkey was used in the comparison of the two radioligands. Each monkey had a minimum 1-month interval for recuperation between experimental days.

D-Amphetamine (Apoteket, Stockholm, Sweden) was administered at four doses (0.1, 0.2, 0.5, and 1.0 mg/kg, expressed relative to the sulfate salt). These doses are similar to those used for PET imaging and microdialysis studies in nonhuman primates (Breier et al., 1997; Laruelle et al., 1997; Moghaddam et al., 1993).

Image analysis

The parametric images of binding potential (BP) and relative blood flow (R_1) were generated from the original reconstructed PET data with a two-parameter multilinear reference tissue model (MRTM2) (Ichise et al., 2003). The R_1 PET image was fused with the BP image using a tool in PMOD Version 2.55 (Pixel-wise modeling computer software, PMOD Group, Zurich, Switzerland). Anatomical regions of interests (ROIs) were manually defined on the fused image for left and right striatum, thalamus, and cerebellum. The MRTM2 model requires a priori estimation of a reference region clearance rate (k_2'), which was estimated using the three-parameter MRTM from striatal and cerebellar ROI activities. To optimize the reliability of k_2' estimation, a weighted (according to ROI size) mean value estimated from the ROI time activity curves (TACs) of the right and left striatum was used. All parametric imaging was performed in PMOD (Mikolajczyk et al., 1998) installed on a PC workstation.

Regional radioactivity was normalized to injected activity and body weight by use of % of standard uptake value [%SUV = (% injection dose/cm^3 brain) × body weight (g)]. The radioactivity in the cerebellum was used as an approximate value for free and nonspecifically bound radioligand concentration in the brain. The time curve for the ratio of radioactivity was calculated for each ROI to the cerebellum. Specific binding to D_2 receptors in striatum was defined as the difference between the total radioactivity concentration in the striatum and the cerebellum.

The change in BP after administration of amphetamine was calculated according to the equation:

$$\text{Change}(\%) = ((BP_{Amphetamine} - BP_{Baseline})/BP_{Baseline}) \times 100$$

Statistical analysis

On data obtained under baseline conditions, a comparison of the two radioligands outcome measure (BP) was assessed by a repeated measured ANOVA (RM ANOVA), with the radioligand as the repeated condition ($n = 8$ for each radioligand). For each radioligand, the between monkey differences at baseline were investigated by one-way analysis of variance (ANOVA), with monkey as group factor ($n = 8$ for each radioligand).

The effect of AMPH on the change in BP for each radioligand was evaluated by a one-way ANOVA. The change in BP for each radioligand was designated as the dependent variable and the main effect assessed for dose, with an interaction between the radioligands and the ligand × dose. In addition, we applied a RM ANOVA to the data, including both radiotracers, and treating each study (study 1 and 2; for each radioligand) as separate observations. In the present study, we have four monkeys with four different doses of AMPH, but each monkey received the same dose of AMPH with both radioligands. Assuming the monkey's baseline values are similar, we treated each monkey as a separate observation when calculating the RM ANOVA. The minimum level of significance was designated as $P < 0.05$. All statistical analyses were performed using SPSS version 13.

The reproducibility and variability of the effects of amphetamine on the change in BP of [^{11}C]raclopride and [^{11}C]MNPA were evaluated. Reproducibility were evaluated by calculating bias (100 × (test − retest/test)) and variability (standard deviation of the bias).

RESULTS

The injected radioactivity and mass dose were similar for the two radioligands in the baseline and pretreatment conditions. For [^{11}C]MNPA, the baseline injection was 54 ± 4 MBq and 0.16 ± 0.14 μg ($n = 8$),

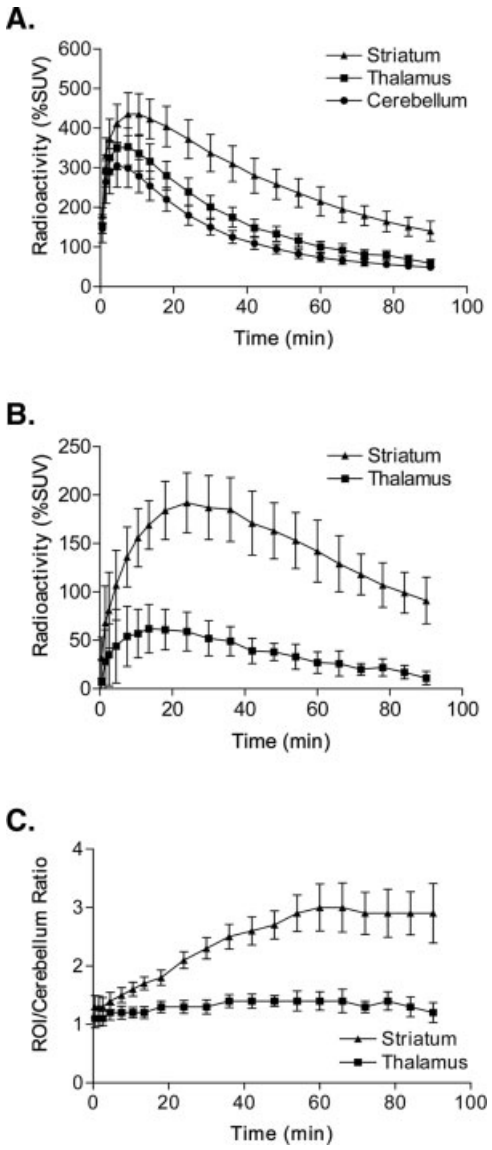

Fig. 1. **A:** Average time course for regional radioactivity (%SUV) in the striatum, thalamus, and cerebellum after i.v. bolus injection of [^{11}C]MNPA at baseline. Values represent (mean ± SD; $n = 8$). **B:** Time course for specific binding (%SUV). **C:** Binding ratios.

and the pretreatment injection was 54 ± 5 MBq and 0.17 ± 0.14 μg ($n = 8$). For [^{11}C]raclopride, the baseline injection was 58 ± 2 MBq and 0.28 ± 0.34 μg ($n = 8$), and the pretreatment injection was 58 ± 3 MBq and 0.23 ± 0.26 μg ($n = 8$). Based on the injected mass dose and D_2 receptor B_{max} of 34 nM (Ginovart et al., 1997), the mean calculated striatal receptor occupancy at baseline for [^{11}C]MNPA and [^{11}C]raclopride was 2.0 and 3.0%, respectively.

Following injection of [^{11}C]MNPA in four monkeys in the baseline condition, activity concentrated in striatum, with lower levels in the thalamus that were still higher than the nonspecific uptake in cerebellum (Fig. 1A). The specific binding in the striatum peaked between 20 and 30 min after injection, and the ratio of the striatum to the cerebellum peaked at 60–70 min post injection at values of 3.0 ± 0.41 ($n = 8$) (Figs. 1B and 1C). [^{11}C]Raclopride had a much higher ratio of specific to nonspecific uptake than [^{11}C]MNPA. Thus, under baseline conditions, [^{11}C]raclopride BP (5.76 ± 0.95, $n = 8$) was significantly higher than that for [^{11}C]MNPA BP (1.31 ± 0.21, $n = 8$) (RM ANOVA $F(1,7) = 118.14$; $P = 0.00$). When comparing the difference between the four monkeys BP values for each radioligand, no significance between subject effects were found for [^{11}C]MNPA or [^{11}C]raclopride ($P > 0.10$; ANOVA).

Amphetamine effects on [^{11}C]MNPA and [^{11}C]raclopride BP

The effect of AMPH on the binding of [^{11}C]MNPA and [^{11}C]raclopride was investigated by administering AMPH i.v. (0.1, 0.2, 0.5, and 1.0 mg/kg) ~20 min before the injection of radioligand. Table I shows BP values of [^{11}C]MNPA and [^{11}C]raclopride under baseline and postamphetamine conditions. AMPH caused a dose-dependent reduction in [^{11}C]MNPA BP of 4% at 0.1, 23% at 0.2, 25% at 0.5, and 46% at 1.0 mg/kg (Figs. 2 and 3A). [^{11}C]Raclopride BP was reduced to a lesser extent by 2% at 0.1, 16% at 0.2, 15% at 0.5, and 23% at 1.0 mg/kg of AMPH (Figs. 2 and 3B). Although the sample size is limited in this study, we performed a preliminary statistical analysis, which should be interpreted with caution. A significant dose effect was observed for both radioligands ($P = 0.004$; ANOVA), demonstrating that AMPH reduced the BP of each radioligand compared with baseline measurements, as shown in Figures 3A and 3B. No statistically significant differences were found when comparing the interactions between the radioligands and the effect of dose of AMPH; ligand × dose ($P = 0.33$; ANOVA). A statistically significant greater change in BP for [^{11}C]MNPA compared with [^{11}C]raclopride was observed ($P = 0.024$; ANOVA). When both radioligands and each study was used as a separate observation in the analysis, no significant effect of dose was observed ($P = 0.11$; RM ANOVA).

Reproducibility of the amphetamine effect

The reproducibility of change in BP after amphetamine administration was evaluated by calculating bias and variability. The change in BP across three doses of AMPH (0.2, 0.5, and 1.0 mg/kg) showed moderate reproducibility with a mean bias of 22% and variability of 29% for [^{11}C]MNPA and a mean bias of 31% and variability of 11% for [^{11}C]raclopride.

Percentage of D2 receptors in the high affinity state

The change in BP ratio ([^{11}C]MNPA ΔBP/[^{11}C]-Raclopride ΔBP) values are given in Table II. Based

TABLE I. Striatal BP values for [^{11}C]MNPA and [^{11}C]raclopride binding at baseline condition and after i.v. administration of amphetamine in four cynomolgus monkeys

	Animal (AMPH dose (mg/kg))											
	A (0.1)			B (0.2)			C (0.5)			D (1.0)		
	Baseline	AMPH	Change %	Baseline	AMPH	Change %	Baseline	AMPH	Change %	Baseline	AMPH	Change %
[^{11}C]MNPA–study 1	1.27	1.21	−5	1.11	0.80	−28	1.61	1.23	−24	0.93	0.38	−59
[^{11}C]MNPA–study 2	1.46	1.40	−4	1.37	1.11	−19	1.33	0.98	−26	1.42	0.95	−33
Mean	1.37	1.31	−4	1.24	0.96	−23	1.47	1.11	−25	1.18	0.67	−46
[^{11}C]raclopride– study 1	6.05	6.17	2	6.11	4.90	−20	6.97	5.80	−17	6.62	4.89	−26
[^{11}C]raclopride– study 2	5.47	5.10	−7	4.63	4.11	−11	4.17	3.65	−13	4.49	3.62	−19
Mean	5.76	5.64	−2	5.37	4.51	−16	5.57	4.72	−15	5.56	4.26	−23

Effect of various doses of amphetamine compared with baseline BP shown as percent change. "Study 1" refers to the first scanning day (e.g., baseline in the morning and AMPH in afternoon). "Study 2" refers to another day in which these two scans were repeated.

on calculations proposed by Narendran et al. (2004) and the assumption that 10% of receptors are occupied by DA at baseline, our results suggest that 61% of the D_2 receptors are configured in the high affinity state. This calculation is based upon the assumption that 10% of the receptors are occupied by DA at baseline, and that extrasynaptic receptors cannot be displaced by stimulant-induced DA release. This 61% in the high affinity state is distributed as follows: 10% occupied by DA at baseline, 23% are synaptic and 28% are extrasynaptic. By this calculation, the remaining receptors (39%) would be in the low affinity state (i.e., 39% = 100 − 61% in the high affinity state) (Fig. 4). This calculation can be understood by considering those receptors that are available to labeling by MNPA versus raclopride, and the measured ratio of amphetamine-induced displacement for raclopride to MNPA is 57%. By the final calculation (Fig. 4), the displaceable (i.e., synaptic) component of [^{11}C]-raclopride binding is 23/(39 + 23 + 28), which equals to 26%; and that for [^{11}C]MNPA binding is 23/(23 + 28), which equals to 45%. Thus, the ratio of % displaced by [^{11}C]raclopride compared with [^{11}C]MNPA is 26/45 = 57%, which is the average ratio we determined in these studies for four doses of AMPH.

DISCUSSION

The present study provides evidence that the agonist radioligand [^{11}C]MNPA is more sensitive than the antagonist radioligand [^{11}C]raclopride to displacement by endogenous DA. Since an antagonist radioligand binds with equal affinity to both high- and low-affinity states, a radiolabeled agonist would be advantageous since it only binds to receptors in the high-affinity state, likely leading to more sensitivity to endogenous DA. At all doses examined, the change in [^{11}C]MNPA BP was ∼50% more sensitive than [^{11}C]raclopride to pharmacological induced increases in synaptic DA. For example, the maximal dose of 1.0 mg/kg of AMPH caused a 46% reduction in the BP of [^{11}C]MNPA, compared with only 23% of [^{11}C]-raclopride. These results agree with those of Cumming et al. (2002) and Narendran et al. (2004), who similarly investigated the effect of AMPH on the binding of agonist and antagonist radioligands in mice and nonhuman primates.

In vivo estimation of the proportion of D_2 receptors in the high-affinity state has been hampered by the lack of available agonist radioligands. In the present study, we estimated the percentage of high-affinity state receptors to be ∼61%, which is similar to the value of 71% previously published by Narendran et al. (2004). However, these estimates are based on two questionable assumptions. The first is that 10% of D_2 receptors are occupied by DA at baseline. In fact, this value could be higher, since others have

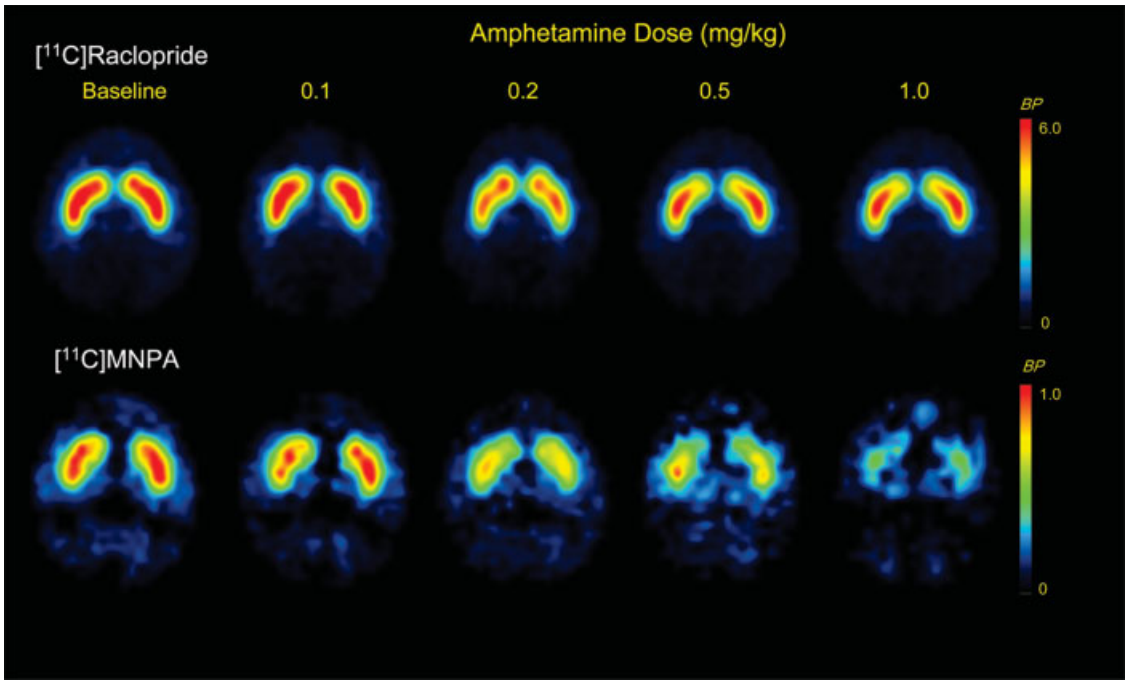

Fig. 2. Parametric images of [^{11}C]raclopride and [^{11}C]MNPA *BP* estimated by MRTM2 at baseline and postamphetamine conditions. Images represent the same monkey for each dose of amphetamine.

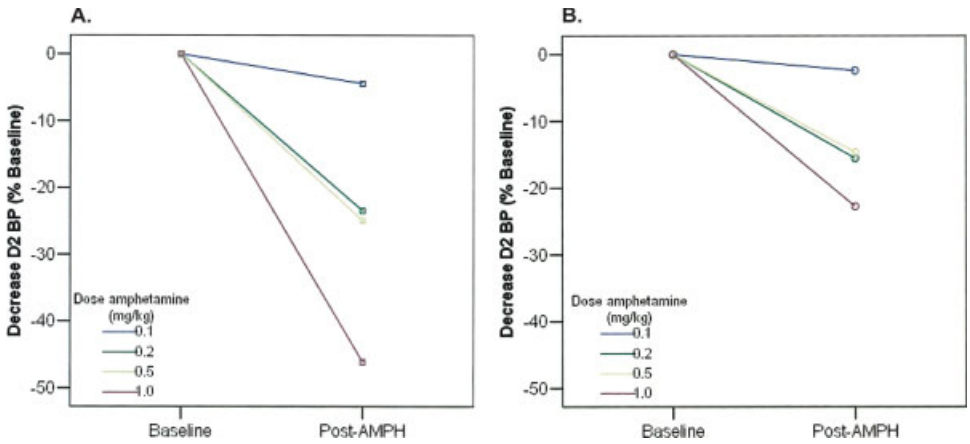

Fig. 3. Effect of an amphetamine challenge on the percentage ΔBP of [^{11}C]MNPA (**A**) and [^{11}C]raclopride (**B**). Each dose of AMPH (0.1, 0.2, 0.5, and 1.0 mg/kg) represents one monkey for both [^{11}C]raclopride and [^{11}C]MNPA. Points represent mean change in *BP* after amphetamine administration compared with baseline.

reported variable results in human and nonhuman primates ranging from 9 to 35% (Abi-Dargham et al., 2000; Ginovart et al., 1997; Laruelle et al., 1997; Verhoeff et al., 2001). However, even if 30% of receptors are occupied by DA at baseline, our calculation increased from 61 to only 70% of receptors in the high affinity state. The second assumption is that some portion of the D_2 receptors cannot be displaced by DA and have been termed extrasynaptic. For example, these receptors could be internalized and capable of being reached by the lipophilic radiotracer but not by the positively charged DA molecule. Further studies with agonist and antagonist radioligands both in vivo and in vitro are likely to refine these estimates. For example, preliminary in vivo saturation binding studies in cynomolgus monkeys with [^{11}C]MNPA and [^{11}C]raclopride have estimated the percentage of D_2 receptor high-affinity state to be around 50% (Finnema et al., 2005b). Thus, both methods (i.e., amphetamine-induced displacement and saturation binding studies) lead to similar estimate that 50–60% of D_2 receptors in four anesthetized primates are in the high affinity state.

The current results have been interpreted in Figure 4 with the understanding that the [^{11}C]raclopride binds to a greater number of D_2 receptors than [^{11}C]MNPA, because the antagonist binds to both the high and low affinity states of the receptor. In fact, there may be other reasons for differences in B'_{max} (available receptor density) between these two trac-

TABLE II. [^{11}C]MNPA to [^{11}C]raclopride ΔBP ratios

Amphetamine dose (mg/kg)	*n*	[^{11}C]MNPA change BP/[^{11}C]raclopride change BP
0.1	2	1.85
0.2	2	1.51
0.5	2	1.70
1.0	2	2.03
Average of all doses		1.77

ers. For example, the ligands may differ in affinity for the monomer and dimer forms of the receptor. It has been demonstrated that D_2 receptors exist either as monomers or dimers in rat and human brain tissue (Ng et al., 1996; Zawarynski et al., 1998). Benzamide D_2 antagonist radioligands bind to both DA D_2 monomer and dimer sites, while radiolabeled spiperone binds only to the monomer sites (Zawarynski et al., 1998). This has lead to discrepancies in reported densities of D_2 receptors in human brain assessed by PET with B_{max} values for [^{11}C]raclopride (25 pmol/ml) and [^{11}C]methylspiperone (15 pmol/ml) in controls and disease state (Farde et al., 1990; Wong et al., 1986). It is possible that [^{11}C]MNPA may bind only to the dimer state of the receptor, considering that the high-affinity state of the receptor is associated with a higher molecular weight of the receptor possibly leading to a lower BP value for the agonist radioligand compared with the antagonist.

Effects of anesthesia

Ketamine produces a dissociative anesthesia mediated by noncompetitive antagonism of the *N*-methyl-D-aspartate (NMDA) receptor. Stimulation of DA release by ketamine has been shown to be selective to prefrontal cortex in rat (Verma and Moghaddam, 1996), with only negligible increases in monkey striatum (Adams et al., 2002). In the present study, a mixture of ketamine and xylazine (2.5 and 1.0 mg/kg, respectively) was administered i.m. every 40 min at baseline and during amphetamine challenge conditions. Ketamine, sometimes at even greater doses, has been found to have no effect on [^{11}C]raclopride binding in striatum of cat (Hassoun et al., 2003) and human brain (Aalto et al., 2002; Kegeles et al., 2002). However, other researchers have reported ketamine-induced reductions of striatal [^{11}C]raclopride uptake in humans (Smith et al., 1998; Vollenweider et al., 2000) and nonhuman primates (Tsukada et al., 2000). The contrasting results of these studies may be related to the dose and manner of administration of ketamine and the radioligand. In conclusion, despite contrasting results it cannot be ruled out that ketamine has some effect on [^{11}C]MNPA and [^{11}C]raclopride binding at both baseline and pretreatment conditions.

Independent of any effect of ketamine to release endogenous DA, anesthesia may also affect the affinity state of the receptor. Seeman and Kapur (2003) have reported that high concentrations of ketamine converted the D_2 receptor from high to low affinity state. If these doses apply to the current studies, then an even higher percentage of D_2 receptors will be in the high affinity state of the awake primate. Finally, it is worth noting that ketamine and AMPH can cause changes in blood flow, but this should have no effect on the measured BP values. As shown by Ichise et al. (2003), MRTM2 calculations of BP are independent of blood flow.

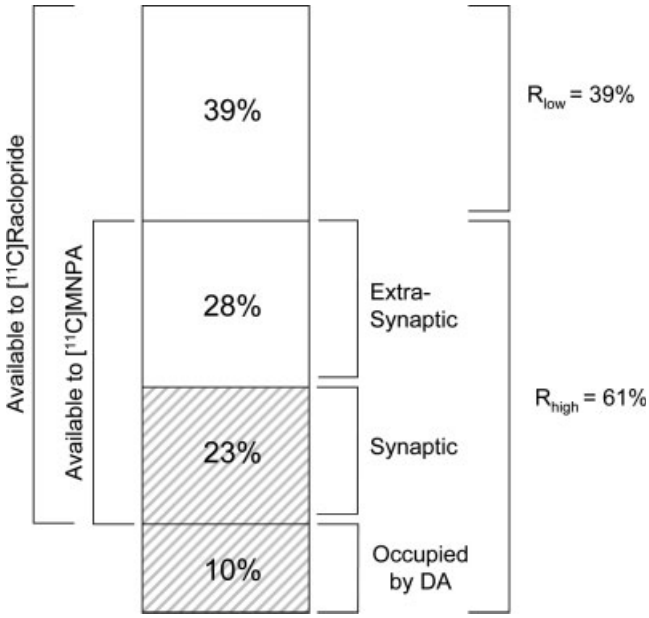

Fig. 4. In vivo estimation of percentage of D_2 receptors in high and low affinity states. Based on calculations proposed by Narendran et al. (2004) and the assumption that 10% of receptors are occupied by DA at baseline, our results suggest that ~61% of the D_2 receptors are configured in the high affinity state and ~39% are in the low affinity state. The two shaded boxes correspond to the proportion of D_2 receptors that are available to occupancy by DA.

DA D_2 receptor desensitization and resensitization

The greater reduction in BP after amphetamine administration for [^{11}C]MNPA compared with [^{11}C]raclopride may also be secondary to agonist-induced receptor internalization. Agonist exposure to G-protein coupled receptors typically causes uncoupling (leading to desensitization), which may then be followed by internalization and subsequent recycling to surface membrane and recoupling to the G-protein (leading to resensitization). The data from PET receptor studies combined with pharmacological challenges to release endogenous transmitter are often interpreted as direct competition of radioligand and transmitter for binding to the receptor. In fact, decreased uptake of radioligand may be the result of the loss of available binding sites by internalization and a subse-

quent loss of appropriate three-dimensional binding to antagonists as well as agonists.

Comparison of PET D_2 agonist radioligands

The results with [^{11}C]MNPA are quite similar to those with [^{11}C]NPA. The baseline striatum/cerebellum ratio was ~3.0 for [^{11}C]MNPA and has been reported as 2.8 for [^{11}C]NPA (Hwang et al., 2005). The mean BP value at baseline estimated by MRTM2 was 1.31 for [^{11}C]MNPA, which is similar to the reported V_3'' value for [^{11}C]NPA of 1.21. The amphetamine-induced reduction of [^{11}C]MNPA BP in the present study are comparable with those previously published by Narendran et al. (2004) with [^{11}C]NPA. The highest dose of AMPH (1.0 mg/kg) examined in both studies, resulted in similar maximal change in BP with a reduction of 59% and 63% for [^{11}C]MNPA and [^{11}C]NPA, respectively. One advantage of [^{11}C]MNPA compared with [^{11}C]NPA is that it is labeled by ^{11}C-methylation, which is a simpler method than ^{11}C-propionyl chloride used for [^{11}C]NPA. In addition, the ^{11}C-methylation gives higher specific radioactivity and lower mass dose at the time of injection.

The in vivo baseline striatal ratio of ~3.0 for [^{11}C]MNPA is almost two times higher compared with [^{11}C]PPHT, [^{11}C]ZYY-339, and [^{18}F]-5-OH-FPPAT with ratios in rhesus monkeys of 1.5, 1.5, and 2.0, respectively (Mukerjee et al., 2004; Shi et al., 2004).

For [^{11}C]MNPA, the mean BP value at baseline estimated by MRTM2 was 1.31, which is lower than that reported for [^{11}C]PHNO of 2.38 in rat and 2.8 in human estimated by SRTM (Ginovart et al., 2005a; McCormick et al., 2005). While the BP value was greater for [^{11}C]PHNO, the change in BP after AMPH was similar, resulting in a maximal change in BP with a reduction of 59% at 1.0 mg/kg (nonhuman primate) and 62% at 2.0 mg/kg (rat) for [^{11}C]MNPA and [^{11}C]PHNO, respectively. A greater change in BP with [^{11}C]PHNO was demonstrated in cat brain, showing a maximal change in BP of 88% after administration of 2.0 mg/kg of AMPH (Ginovart et al., 2005b). The highest BP values reported in human brain for [^{11}C]PHNO was 3.38 in the globus pallidus. The globus pallidus is a region of the brain known to contain moderate to high densities of DA D_3 receptor, but low densities of D_2. The observation of high displacement in cat brain and high uptake in globus pallidus is consistent with early pharmacological binding studies of CHO cell lines expressing hD_3 and hD_2 receptors. PHNO was reported to have a higher affinity for the hD_3 compared with hD_2 receptor, 8.5 and 0.16 K_i (nM) with a hD_2/hD_3 ratio of 53, compared with NPA 0.12 and 0.21 K_i (nM) with a hD_2/hD_3 ratio of 0.57 (Freedman et al., 1994). The lack of suitable selective D_3 compounds that can cross the blood-brain barrier makes it difficult to quantify the exact proportion of binding to D_3 receptors. The DA subtype selectivity of these agonist radioligands needs to be further assessed.

In conclusion, [^{11}C]MNPA is a promising radioligand for PET-imaging of the high affinity state of D_2 receptors in the primate brain. Stimulation of DA release with amphetamine caused marked decreases in BP of [^{11}C]MNPA in a dose-dependent fashion, thus demonstrating that the radioligand is sensitive to the endogenous DA concentration. A DA agonist radioligand may lead to a further understanding of the pathophysiology of disorders associated with DA dysfunction such as schizophrenia and Parkinson's disease. Imaging studies utilizing [^{11}C]MNPA, which has greater sensitivity to changes in DA function, may be able to assess disease progression, therapeutic treatment, and the efficacy of neuroprotective agents more effectively.

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REFERENCES

Aalto S, Hirvonen J, Kajander J, Scheinin H, Nagren K, Vilkman H, Gustafsson L, Syvalahti E, Hietala J. 2002. Ketamine does not decrease striatal dopamine D2 receptor binding in man. Psychopharmacology (Berl) 164:401–406.

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M. 2000. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci USA 97:8104–8109.

Adams BW, Bradberry CW, Moghaddam B. 2002. NMDA antagonist effects on striatal dopamine release: microdialysis studies in awake monkeys. Synapse 43:12–18.

Anand A, Verhoeff P, Seneca N, Zoghbi SS, Seibyl JP, Charney DS, Innis RB. 2000. Brain SPECT imaging of amphetamine-induced dopamine release in euthymic bipolar disorder patients. Am J Psychiatr 157:1108–1114.

Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A, Weinberger DR, Weisenfeld N, Malhotra AK, Eckelman WC, Pickar D. 1997. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci USA 94:2569–2574.

Carson RE, Breier A, de Bartolomeis A, Saunders RC, Su TP, Schmall B, Der MG, Pickar D, Eckelman WC. 1997. Quantification of amphetamine-induced changes in [^{11}C]raclopride binding with continuous infusion. J Cereb Blood Flow Metab 17:437–447.

Creese I, Sibley DR, Leff SE. 1984. Agonist interactions with dopamine receptors: focus on radioligand-binding studies. Fed Proc 43:2779–2784.

Cumming P, Wong DF, Gillings N, Hilton J, Scheffel U, Gjedde A. 2002. Specific binding of [(11)C]raclopride and *N*-[(3)H]propyl-norapomorphine to dopamine receptors in living mouse striatum: occupancy by endogenous dopamine and guanosine triphosphate-free G protein. J Cereb Blood Flow Metab 22:596–604.

Cumming P, Gillings NM, Jensen SB, Bjarkam C, Gjedde A. 2003. Kinetics of the uptake and distribution of the dopamine D(2,3) agonist (*R*)-*N*-[1-(11)C]*n*-propylnorapomorphine in brain of healthy and MPTP-treated Gottingen miniature pigs. Nucl Med Biol 30: 547–553.

de la Fuente-Fernandez R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. 2001. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. Science 293:1164–1166.

Farde L, Hall H. 1992. Positron emission tomography—examination of chemical transmission in the living human brain. Development of radioligands. Arzneimittelforschung 42:260–264.

Farde L, Wiesel FA, Hall H, Halldin C, Stone-Elander S, Sedvall G. 1987. No D2 receptor increase in PET study of schizophrenia. Arch Gen Psychiatr 44:671–672.

Farde L, Wiesel FA, Stone-Elander S, Halldin C, Nordstrom AL, Hall H, Sedvall G. 1990. D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron emission tomography study with [^{11}C]raclopride. Arch Gen Psychiatr 47:213–219.

Finnema SJ, Seneca N, Farde L, Shchukin E, Sovago J, Gulyas B, Wikstrom HV, Innis RB, Neumeyer JL, Halldin C. 2005a. A preliminary PET evaluation of the new dopamine D2 receptor agonist [^{11}C]MNPA in cynomolgus monkey. Nucl Med Biol 32:353–360.

Finnema SJ, Seneca N, Farde L, Gulyás B, Bang-Andersen B, Wikström HV, Innis RB, Halldin C. 2005b. Scatchard analysis of the D2 receptor agonist [^{11}C]MNPA in the monkey brain using PET. Eur J Nucl Med 32:SB2:293.

Freedman SB, Patel S, Marwood R, Emms F, Seabrook GR, Knowles MR, McAllister G. 1994. Expression and pharmacological characterization of the human D3 dopamine receptor. J Pharmacol Exp Ther 268:417–426.

Gao YG, Baldessarini RJ, Kula NS, Neumeyer JL. 1990. Synthesis and dopamine receptor affinities of enantiomers of 2-substituted apomorphines and their *N*-*n*-propyl analogues. J Med Chem 33:1800–1805.

George SR, Watanabe M, Di Paolo T, Falardeau P, Labrie F, Seeman P. 1985. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. Endocrinology 117:690–697.

Ginovart N, Farde L, Halldin C, Swahn CG. 1997. Effect of reserpine-induced depletion of synaptic dopamine on [^{11}C]raclopride binding to D2-dopamine receptors in the monkey brain. Synapse 25:321–325.

Ginovart N, Willeit M, Kapur S, Hussey D, Garcia A, Houle S, Wilson A. 2005a. First evaluation of the dopamine-D2 receptors using the agonist radiotracer [^{11}C]-(+)-PHNO in healthy human volunteers. SNM 05:38.

Ginovart N, Wilson A, Willeit M, Singh K, Hussey D, Garcia A, Houle S, Kapur S. 2005b. The D2 agonist radiotracer [^{11}C]-(+)-PHNO shows a marked increase in sensitivity to amphetamine challenges when compared to [^{11}C]raclopride in the cat brain. Brain PET. 05:BP–25.

Hassoun W, Le Cavorsin M, Ginovart N, Zimmer L, Gualda V, Bonnefoi F, Leviel V. 2003. PET study of the [^{11}C] raclopride binding in the striatum of the awake cat: effects of anaesthetics and role of cerebral blood flow. Eur J Nucl Med Mol Imaging 30:141–148.

Hwang DR, Narendran R, Laruelle M. 2005. Positron-labeled dopamine agonists for probing the high affinity states of dopamine subtype 2 receptors. Bioconjug Chem 16:27–31.

Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, Suhara T, Suzuki K, Innis RB, Carson RE. 2003. Linearized reference tissue parametric imaging methods: application to [^{11}C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 23:1096–1112.

Innis RB, Malison RT, al-Tikriti M, Hoffer PB, Sybirska EH, Seibyl JP, Zoghbi SS, Baldwin RM, Laruelle M, Smith EO, Charney D, Heninger G, Elsworth J, Roth R. 1992. Amphetamine-stimulated dopamine release competes in vivo for [123I]IBZM binding to the D2 receptor in nonhuman primates. Synapse 10:177–184.

Karlsson P, Farde L, Halldin C, Swahn CG, Sedvall G, Foged C, Hansen KT, Skrumsager B. 1993. PET examination of [^{11}C]NNC 687 and [^{11}C]NNC 756 as new radioligands for the D1-dopamine receptor. Psychopharmacology (Berl) 113:149–156.

Kegeles LS, Martinez D, Kochan LD, Hwang DR, Huang Y, Mawlawi O, Suckow RF, Van Heertum RL, Laruelle M. 2002. NMDA antagonist effects on striatal dopamine release: positron emission tomography studies in humans. Synapse 43:19–29.

Kohler C, Fuxe K, Ross SB. 1981. Regional in vivo binding of [3H]*N*-propylnorapomorphine in the mouse brain. Evidence for labelling of central dopamine receptors. Eur J Pharmacol 72:397–402.

Langer O, Någren K, Dolle F, Lundkvist C, Sandell J, Swahn C, Vaufrey F, Crouzel C, Maziere B, Halldin C. 1999. Precursor synthesis and radiolabelling of the dopamine. J Labelled Comp Rad 42:1183–1193.

Laruelle M. 2000. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J Cereb Blood Flow Metab 20:423–451.

Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, Baldwin RM, Charney DS, Hoffer PB, Kung HF, Innis RB. 1995. SPECT imaging of striatal dopamine release after amphetamine challenge. J Nucl Med 36:1182–1190.

Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seibyl JP, Krystal JH, Charney DS, Innis RB. 1996. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci USA 93:9235–9240.

Laruelle M, Iyer RN, al-Tikriti MS, Zea-Ponce Y, Malison R, Zoghbi SS, Baldwin RM, Kung HF, Charney DS, Hoffer PB, Innis RB, Bradberry CW. 1997. Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. Synapse 25:1–14.

McCormick P, Ginovart N, Kapur S, Hussey D, Garcia A, Seeman P, Houle S, Wilson A. 2005. [^{11}C]-(+)-PHNO, A potent dopamine D2 agonist radiotracer is sensitive to manipulations of endogenous dopamine in rat. SNM 05:202.

Mikolajczyk K, Szabatin M, Rudnicki P, Grodzki M, Burger C. 1998. A JAVA environment for medical image data analysis: initial application for brain PET quantitation. Med Inform (Lond) 23:207–214.

Moghaddam B, Berridge CW, Goldman-Rakic PS, Bunney BS, Roth RH. 1993. In vivo assessment of basal and drug-induced dopamine release in cortical and subcortical regions of the anesthetized primate. Synapse 13:215–222.

Mukherjee J, Narayanan TK, Christian BT, Shi B, Yang ZY. 2004. Binding characteristics of high-affinity dopamine D2/D3 receptor agonists, 11C-PPHT and 11C-ZYY-339 in rodents and imaging in non-human primates by PET. Synapse 54:83–91.

Narendran R, Hwang DR, Slifstein M, Talbot PS, Erritzoe D, Huang Y, Cooper TB, Martinez D, Kegeles LS, Abi-Dargham A, Laruelle M. 2004. In vivo vulnerability to competition by endogenous dopamine: comparison of the D2 receptor agonist radiotracer (−)-*N*-[^{11}C]propyl-norapomorphine ([^{11}C]NPA) with the D2 receptor antagonist radiotracer [^{11}C]-raclopride. Synapse 52:188–208.

Neumeyer J, Gao Y, Kula N, Baldessarini R. 1990. Synthesis and Dopamine receptor affinityof (*R*)-(-)-Fluoro-*N*-*n*-propylnorapmorphine: a highly potent and selective dopamine D2 agonist. J Med Chem 33:3122–3124.

Ng GY, O'Dowd BF, Lee SP, Chung HT, Brann MR, Seeman P, George SR. 1996. Dopamine D2 receptor dimers and receptor-blocking peptides. Biochem Biophys Res Commun 227:200–204.

Parsey RV, Oquendo MA, Zea-Ponce Y, Rodenhiser J, Kegeles LS, Pratap M, Cooper TB, Van Heertum R, Mann JJ, Laruelle M. 2001. Dopamine D(2) receptor availability and amphetamine-induced dopamine release in unipolar depression. Biol Psychiatr 50:313–322.

Ross SB, Jackson DM. 1989. Kinetic properties of the in vivo accumulation of 3H-(-)-*N*-*n*-propylnorapomorphine in mouse brain. Naunyn Schmiedebergs Arch Pharmacol 340:13–20.

Seeman P, Kapur S. 2003. Anesthetics inhibit high-affinity states of dopamine D2 and other G-linked receptors. Synapse 50:35–40.

Seeman P, Watanabe M, Grigoriadis D, Tedesco JL, George SR, Svensson U, Nilsson JL, Neumeyer JL. 1985. Dopamine D2 receptor binding sites for agonists. A tetrahedral model. Mol Pharmacol 28:391–399.

Shi B, Narayanan TK, Christian BT, Chattopadhyay S, Mukherjee J. 2004. Synthesis and biological evaluation of the binding of dopamine D2/D3 receptor agonist, (*R*,*S*)-5 hydroxy-2-(*N*-propyl-*N*-(5′-(18)F-fluoropentyl)aminotetralin((18)F-5-OH-FPPAT) in rodents and nonhuman primates. Nucl Med Biol 31:303–311.

Sibley DR, Creese I. 1983. Regulation of ligand binding to pituitary D-2 dopaminergic receptors. Effects of divalent cations and functional group modification. J Biol Chem 258:4957–4965.

Sibley DR, Mahan LC, Creese I. 1983. Dopamine receptor binding on intact cells. Absence of a high-affinity agonist-receptor binding state. Mol Pharmacol 23:295–302.

Smith GS, Schloesser R, Brodie JD, Dewey SL, Logan J, Vitkun SA, Simkowitz P, Hurley A, Cooper T, Volkow ND, Cancro R. 1998. Glutamate modulation of dopamine measured in vivo with positron emission tomography (PET) and ^{11}C-raclopride in normal human subjects. Neuropsychopharmacology 18:18–25.

Tedroff J, Pedersen M, Aquilonius SM, Hartvig P, Jacobsson G, Langstrom B. 1996. Levodopa-induced changes in synaptic dopamine in patients with Parkinson's disease as measured by [^{11}C]raclopride displacement and PET. Neurology 46:1430–1436.

Tsukada H, Harada N, Nishiyama S, Ohba H, Sato K, Fukumoto D, Kakiuchi T. 2000. Ketamine decreased striatal [(11)C]raclopride binding with no alterations in static dopamine concentrations in the striatal extracellular fluid in the monkey brain: multiparametric PET studies combined with microdialysis analysis. Synapse 37:95–103.

van der Werf JF, Sebens JB, Vaalburg W, Korf J. 1983. In vivo binding of *N*-*n*-propylnorapomorphine in the rat brain: regional localization, quantification in striatum and lack of correlation with dopamine metabolism. Eur J Pharmacol 87:259–270.

Verhoeff NP, Kapur S, Hussey D, Lee M, Christensen B, Psych C, Papatheodorou G, Zipursky RB. 2001. A simple method to measure baseline occupancy of neostriatal dopamine D2 receptors by dopamine in vivo in healthy subjects. Neuropsychopharmacology 25:213–223.

Verma A, Moghaddam B. 1996. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. J Neurosci 16:373–379.

Vollenweider FX, Vontobel P, Oye I, Hell D, Leenders KL. 2000. Effects of (*S*)-ketamine on striatal dopamine: a [^{11}C]raclopride PET study of a model psychosis in humans. J Psychiatr Res 34:35–43.

Volkow ND, Wang GJ, Fowler JS, Logan J, Schlyer D, Hitzemann R, Lieberman J, Angrist B, Pappas N, MacGregor R, Burr G, Cooper T, Wolf A. 1994. Imaging endogenous dopamine competition with [^{11}C]raclopride in the human brain. Synapse 16:255–262.

Wienhard K, Dahlbom M, Eriksson L, Michel C, Bruckbauer T, Pietrzyk U, Heiss WD. 1994. The ECAT EXACT HR: performance of a new high resolution positron scanner. J Comput Assist Tomogr 18:110–118.

Wilson AA, McCormick P, Kapur S, Willeit M, Garcia A, Hussey D, Houle S, Seeman P, Ginovart N. 2005. Radiosynthesis and evaluation of [^{11}C]-(+)-4-propyl 3,4,4a,5,6,10b-hexahydro-2H-naphtho [1,2-b][1,4]oxazin-9-ol as a potential radiotracer for in vivo imaging of the dopamine D2 high-affinity state with positron emission tomography. J Med Chem 48:4153–4160.

Wong DF, Wagner HN, Jr., Tune LE, Dannals RF, Pearlson GD, Links JM, Tamminga CA, Broussolle EP, Ravert HT, Wilson AA, et al. 1986. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naïve schizophrenics. Science 234: 1558–1563.

Zawarynski P, Tallerico T, Seeman P, Lee SP, O'Dowd BF, George SR. 1998. Dopamine D2 receptor dimers in human and rat brain. FEBS Lett 441:383–386.